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DEVELOPMENT AND JUVENILE GROWTH OF THE SEA ANEMONE, *TEALIA CRASSICORNIS*

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The development of *Tealia* (= *Urticina*) *crassicornis* (Müller, 1776) has been studied by Appellöf (1900) who reported that in Europe this species releases its gametes freely into the sea and the larval development is independent of the adult. Hand (1955) reported this species as being a brooder. In this paper we present information showing that *T. crassicornis* in the Friday Harbor area has a mode of development similar to that described by Appellöf (1900). We have followed the embryonic and larval development by histological techniques and although our results conform largely to those of Appellöf, we differ from him in several interpretations of major importance. We have successfully reared the larvae to metamorphosis; the young anemones in our laboratory are now 18 months old. Our findings on the induction of larval settlement by worm tubes and on the effect of feeding on juvenile growth should also prove to be significant in anthozoan biology.

Materials and Methods

Tealia crassicornis, collected from the San Juan Island area and kept in the sea water tanks at the Friday Harbor Laboratories, spawned several times during the springs of 1970 and 1971. The eggs and sperm were collected as they were released. The eggs were put into covered culture dishes containing 500 ml of sea water, and a small amount of sperm suspension introduced. After approximately one half hour the eggs were rinsed free of sperm. The water was subsequently changed periodically. Development was allowed to continue in these dishes at approximately 12° C. After being kept four months in the culture dishes some of the juveniles were removed and placed on glass microscope slides. Once they attached, the slides were placed in vertical holders in a "Plankton-Kreisel" (Greve, 1968) as modified by Roosen-Runge (1970). The young anemones in the dishes and the "Kreisel" were fed *Artemia* during the first five months. Starting with the sixth month young anemones in the "Kreisel" were fed pieces of mussel or shrimp meat, while those in the dishes were starved.

For histological preparations, individuals at various stages of development were fixed in Bouin's fluid and embedded in paraffin. Serial sections were cut at 5 μ thickness and stained with Heidenhain's hematoxylin and Orange G.

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Spawning

On four occasions in May and June, 1970, and once in April, 1971, animals were seen to spawn in the laboratory. At least one of each sex spawned every time, and thus all eggs which were allowed to float in the tank were fertilized. In all cases, animals spawned in the morning between 8 and 11 o'clock.

When spawning, the tentacles and the column of the animal were fully extended. Strings of mucus containing gametes were expelled gradually from the mouth, in the manner of undigested food particles being ejected. Eggs, immediately after being expelled from the mouth, attached to each other to form small clusters. They tended to sink, but fell apart before reaching the bottom of the tank, and began to float on the surface of the water. Sperm, on the other hand, became suspended soon after being discharged from the mouth.

While spawning, animals remain still and appear to be somewhat insensitive to mechanical disturbance. For example, probing of a non-spawning individual always causes the retraction of tentacles, but when eggs are collected among the tentacles by using a basting syringe the animal rarely reacts.

Gametes

When expelled, the egg is spherical, yellow or tan in color, and measures from 500 to 700 μ in diameter. It flattens into a pancake shape when placed on a slide and the egg membrane can be easily ruptured in handling, allowing a stream of cytoplasm to escape. The surface of the egg bears numerous spines (Fig. 1), a characteristic of many anthozoan eggs. The spines in this species are relatively blunt, and are 25 μ in length. Meiotic division must have been completed before spawning, as neither germinal vesicle nor polar bodies were observed.

Histological preparation shows that there is a thin (5 μ) cortical layer of fine and dense cytoplasm without yolk platelets. Beneath this layer, there are a number of basophilic, presumably cortical granules, otherwise the oöplasm consists of yolk platelets of two kinds: lipid yolk which become vacuoles (5 μ diameter) after fixation in Bouin's Solution and paraffin sectioning, and small protein yolk granules, 2.5 μ in diameter (Fig. 15).

The sperm of this species are unique, in that not all of them are mature when discharged. In many of them there is still considerable excess cytoplasm housing the nucleus and the middle piece which is attached to a dark body of unknown nature. These two components are seen moving about in the cytoplasm; they may either be separated from each other (Fig. 4) or clumped (Fig. 3). The tail in the meantime beats vigorously. The sperm head is more or less oval in shape, measuring 2×1.5 μ in size, and the tail is 60 μ long (Fig. 2). It is not certain whether or not the immature sperm are functional, but the discharge of these sperm is consistent in all the cases we have examined.

Cleavage

We were puzzled when first examining the eggs superficially in culture dishes, as we could not see any sign of cleavage furrows, even 24 hours after insemination. We discovered later that the cleavage is meroblastic and the first few cleavages

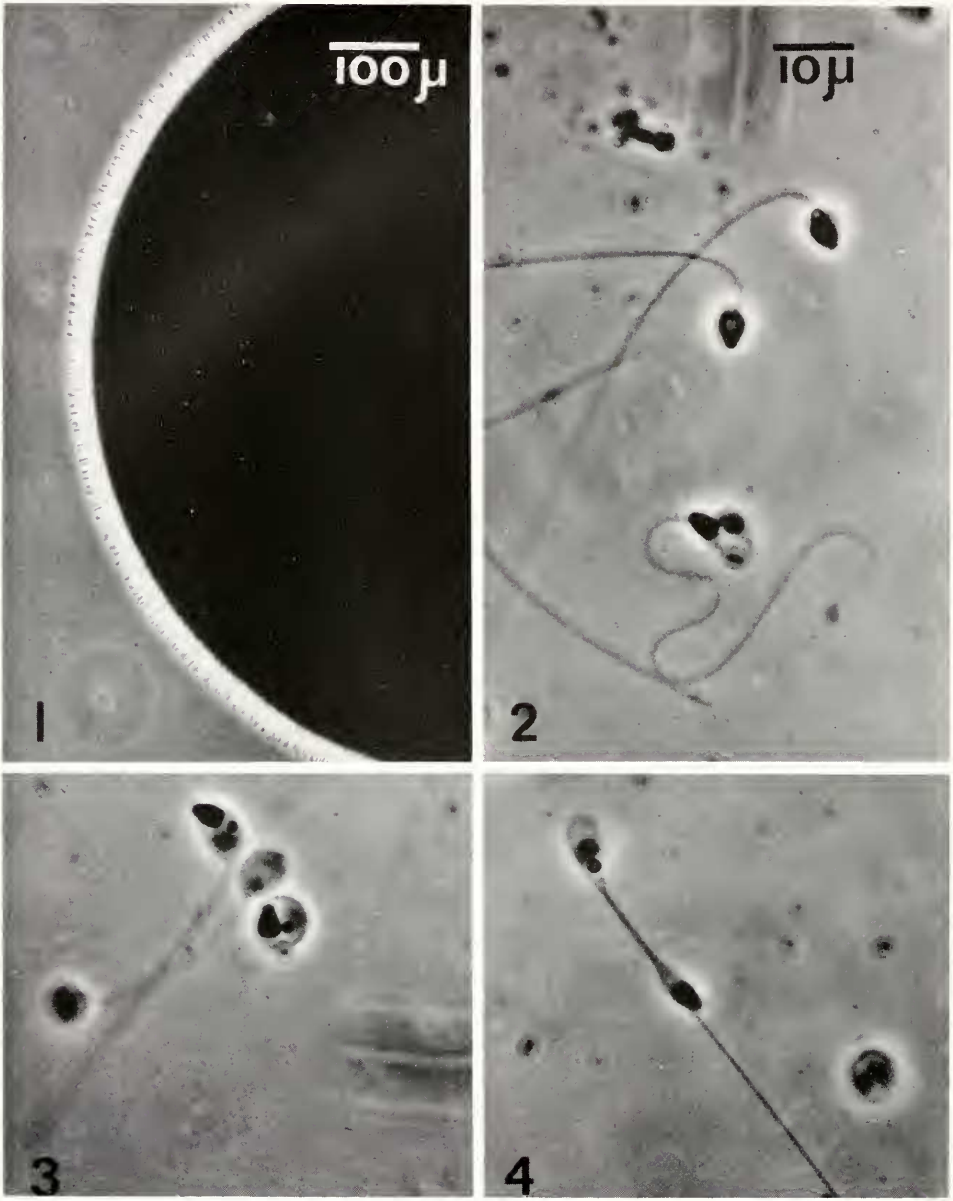


FIGURE 1. Portion of a freshly spawned egg, showing the spiny surface, phase contrast.

FIGURE 2. Mature spermatozoa, phase contrast.

FIGURE 3. Immature spermatozoa, showing the head and the middle piece are clumped together in an excessive amount of cytoplasm, phase contrast.

FIGURE 4. Immature spermatozoa, showing the head and the middle piece are separated, phase contrast.

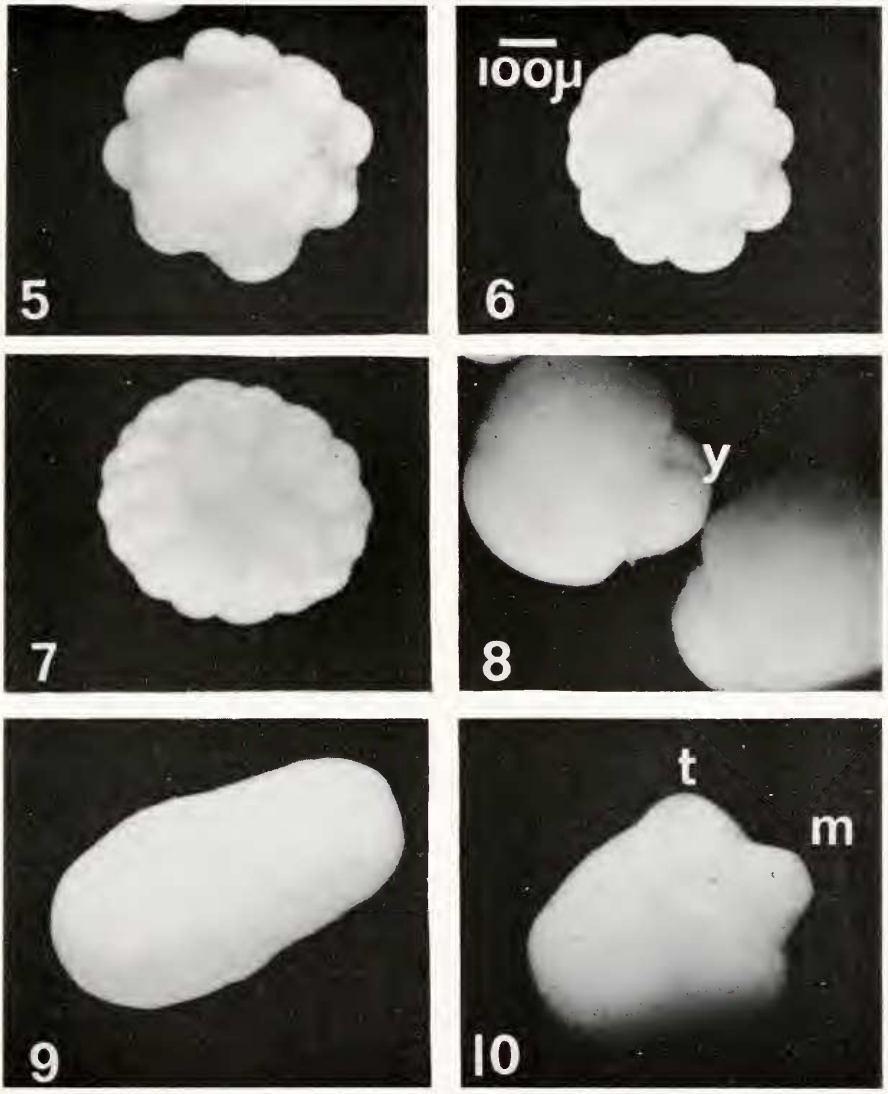


FIGURE 5. Vegetal view of early cleavage stage, showing the superficial nature of cleaving furrows.

FIGURE 6. Animal view of early cleavage stage.

FIGURE 7. Animal view of late cleavage stage.

FIGURE 8. Gastrula, showing a bulged "yolk plug" (y) at vegetal end.

FIGURE 9. Planula, apical end at the left hand side.

FIGURE 10. Metamorphosing planula, showing the protruded mouth (m) and the developing tentacle bud (t).

are confined to the animal pole which is heavier and floats toward the bottom of the dish. Further examinations of living embryos and subsequent histological studies confirmed that the nucleus divided several times before the cell membrane

began to form, first at the animal pole, then at the vegetal pole, resulting in incomplete small blastomeres at the animal half (Figs. 6 and 7) and large blastomeres at the vegetal half (Fig. 5). The result of such superficial cleavage is a blastula with a single layer of poorly defined cells on the surface and a mass of uncleaved yolk in the center (Figs. 16 and 17). No blastocoel ever existed. The outer layer of the superficial cells consists mostly of the fine and basophilic cytoplasm of the cortex (Figs. 16, 17). After histological preparation, the surface spines are clumped together and reduced to 11 μ in length (Figs. 15, 17).

Endoderm formation (gastrulation)

In most of the known cases, gastrulation in anthozoa is accomplished by invagination; hence, the formation of endoderm is by emboly (Mergner, 1971) as far as we can ascertain. In the present species (as in *Halcampa* (Nyholm, 1949) and many of the Hydrozoa) the endoderm appears to arise by multipolar ingression. At gastrula stage, some cells must have migrated into the interior to establish two germ layers: the ectoderm and endoderm. Because of the presence of the yolk mass at this stage, the endoderm is difficult to define in histological preparations. Furthermore, these cells do not form a continuous layer until the planula stage and before that the endoderm cells are scattered with rather ill-defined boundaries.

The gastrulation is also marked by the formation of a structure, similar in appearance to the yolk plug of an amphibian gastrula, at the vegetal pole (Fig. 8). The formation of this "yolk plug" is apparently by the process of epiboly. At this time the embryo begins to elongate along the animal-vegetal axis to become ovoid in outline. The "yolk plug" was large and protruded at the beginning, but soon decreased in size owing to the rapid proliferation of the ectoderm cells of the animal half. In addition, yolky material is being absorbed, leaving the blastopore open as the mouth (Fig. 20). At this stage, the surface spines have disappeared, cilia have developed and the embryo becomes free-swimming. This is then a young planula larva.

Planula

The planula is cone-shaped (Fig. 9), 530 × 750 μ in size, swims with the apical end forward and spends most of the time swimming close to the bottom of the dish. There is no apical tuft as found in many other anthozoan planulae but an invagination (Fig. 18) at the apical end is consistently found in serial sections of the late gastrulae. The apical end of the planula stage no longer shows the invagination, but a group of cells in this area have clearer cytoplasm and lack the basophilic apices characteristic of the cells of the general ectoderm. Histological sections show that the ectoderm is a layer of simple columnar epithelium, and the cells show a considerable degree of differentiation: nematocysts, secretory cells, and spindle-shaped sensory cells (Fig. 19). The endoderm is a layer of simple cuboidal epithelium and is still very rich in yolk granules. Contractile elements have appeared in the endoderm of the body wall and mesenteries, hence the ability of the larvae to change shape. The central mass of yolk, left from earlier stages, is being absorbed, thus the larva becomes hollow establishing the gastrovascular cavity. The tissue which has been pushed inward around the "yolk plug" is now

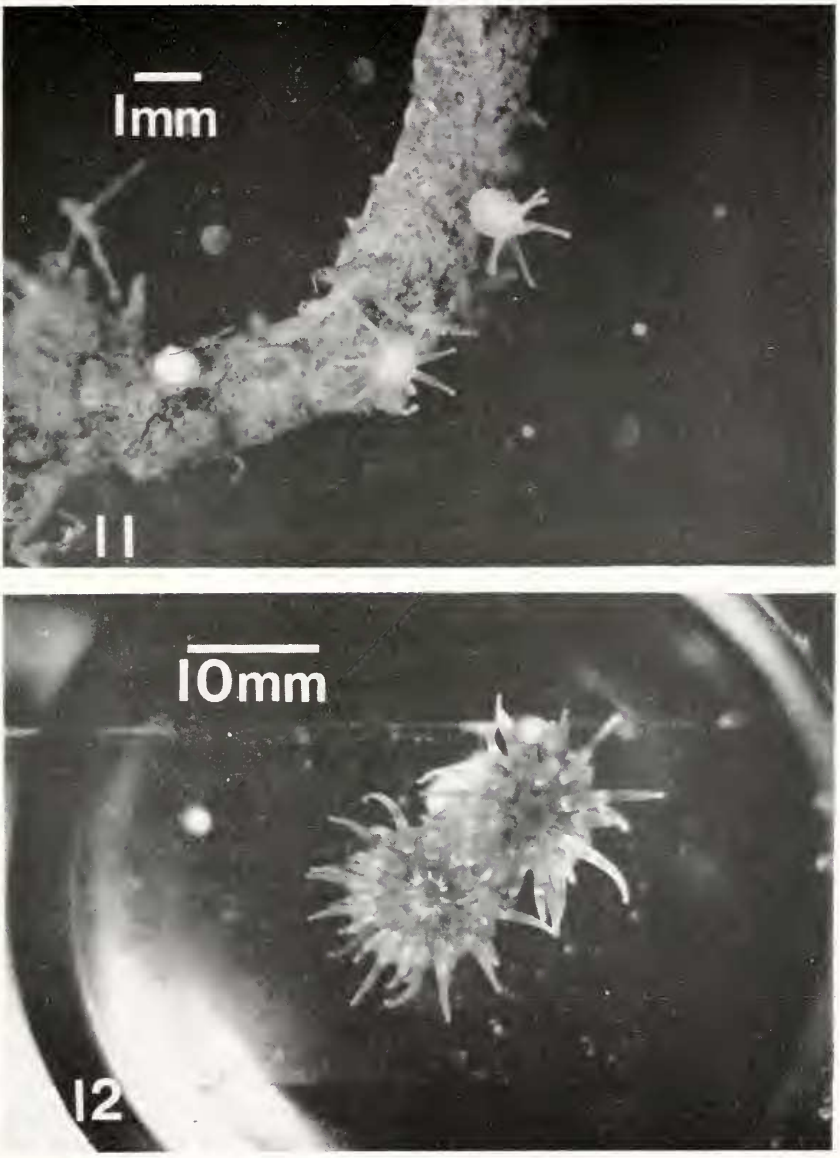


FIGURE 11. Young anemone with 8–12 tentacles, one month after settlement on the *Phyllochaetopterus* tubes.

FIGURE 12. One year old anemone when fed in the laboratory.

the pharynx (Fig. 20). Eight primary septa begin to develop simultaneously, but there is as yet no sign of development of tentacles.

The larva is now generally sticky and can temporarily attach to the surfaces of the culture dishes. Although secretory cells and nematocysts are found distributed throughout the ectoderm, planula larvae were seen only to make contact

on the substratum with the apical end. It is difficult at this stage to pick them up without their sticking to the pipette. They are now nine days old and presumably ready to settle. The chronological development is summarized in Table I.

Effect of substrata on larval settlement

To test the effect of substratum on larval settlement, we set up four finger bowls, each with 150 ml of sea water, and added: (a). *Phyllochaetopterus* sp. (a polychaete worm) tubes, a substratum which has been effective in inducing the larvae of the starfish, *Mediaster aequalis,* to metamorphose (Birkeland, Chia and Strathmann, 1971); (b). some tubes of *Sabellaria cementaria,* another polychaete worm, a substratum used by Long (1964) to induce brachiopod larvae metamorphosis; (c). three slides coated with wax known to induce ascidian

TABLE I

Chronology of larval development

Time (Days)	Developmental events
1	Fertilization; superficial cleavage up to 16-cell stage
2	Blastula; spiny membrane evident
3	Gastrula; formation of "yolk plug"; ciliated; free swimming; spiny membrance still evident
4–5	Gastrula; "yolk plug" disappears; blastopore as a small indentation; spiny membrane disappears.
6	Post-gastrula or young planula; elongated, 500 × 750 μ in size; blastopore becoming the mouth; apical organ formed by invagination
7–9	Planula, more elongated
10	Planula; becoming sticky; begin to make contact with substratum; able to change shape
11	Settlement when *Phyllochaetopterus* or *Sabellaria* tubes are present; newly settled larvae, when extended, measure: diameter of pedal disk = 0.6 mm, length of column = 0.8 mm; 8 septa appeared
17	Young anemone with 4 tentacles
21	Young anemone with 8 tentacles; body wall translucent white; begins to feed
27	Settlement in dishes with sea water only or with small stones and wax-coated slides

larvae to metamorphose, along with a few small stones; and (d). more sea water to serve as control. In each dish we placed 20 larvae from the same spawn which were 10 days old. All of those in dish (a) settled within three days; of those in dish (b), only 11 settled in three days, the rest settled within 10 days; and those in dishes (c) and (d) did not begin to settle until 17 days later, but eventually all settled within the second month. Thus, both *Phyllochaetopterus* and *Sabellaria* tubes can induce the settlement of planula larvae; the *Phyllochaetopterus* being more effective. But the presence of such a substratum is not essential, since planulae in glass dishes will also settle, although after a delay of 17 days. The settlement on these worm tubes is not permanent; they can detach themselves from the substratum and resettle on the glass of the culture dishes.

The first 4 tentacles begin to appear 7 days after settlement and the second 4 tentacles appear 5 days later. At this time, when the young anemones have had 8

tentacles, they begin to react to food such as *Artemia,* mussel or clam meat. The body wall is translucent and whitish in color. The young anemones when fully extended average 0.6 mm in diameter and 0.8 mm in height, and the tentacles are 1 mm long (Fig. 11).

It is interesting to note that the planula larvae which are not settled in dishes (c) and (d) begin to develop tentacles when they are 20 days old; this is only a 3-day delay compared with those settled on worm tubes in dishes (a) and (b). It seems that although the larval settlement is greatly enhanced by environmental factors, such as substratum, the development of tentacles is more or less fixed in a certain age and independent of settling.

Growth of the juvenile anemones

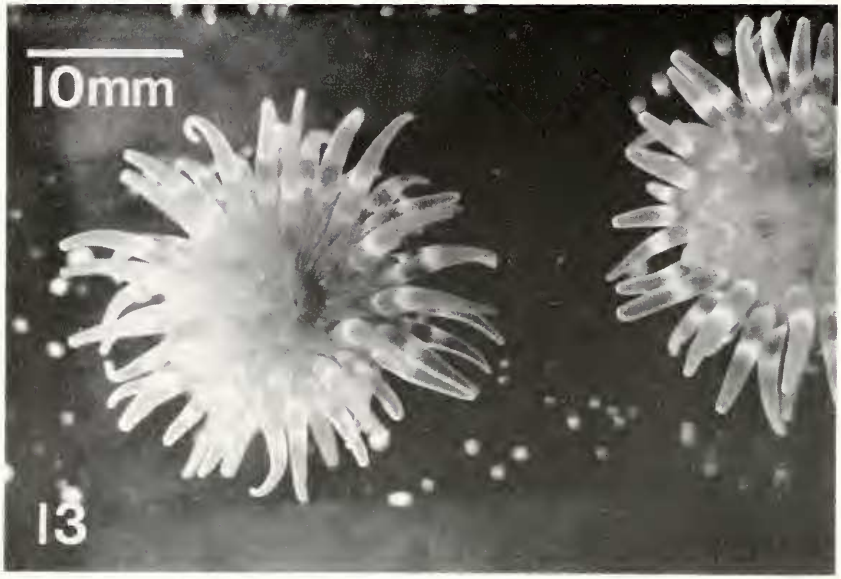
At two months old the anemones measured 0.8 mm across the oral disc and had 12 tentacles averaging 1 mm in length. By the beginning of the fifth month the diameter of the oral disc had increased to an average of 1.7 mm and the anemones had 12 to 16 tentacles averaging 2.1 mm in length. At this time some of the juvenile anemones were transferred to glass slides and put into the "Plankton-Kreisel." Both groups of anemones were fed *Artemia* biweekly until the end of the fifth month. From the beginning of the sixth month the anemones in the "Plankton-Kreisel" were fed mussel or shrimp meat once every month and after the tenth month the feeding was increased to weekly. On the other hand, the anemones in the culture dishes received periodic changes of water but no food. During the tenth month the fed anemones in the "Kreisel" began to develop dark colored bands on their tentacles and oral discs, and their columns became orange in color. The non-fed anemones in the culture dishes did not change.

At one year of age the large fed anemones had reached 10.0 mm in diameter across the oral disc and had 30 to 35 tentacles averaging 6.5 mm in length (Fig. 12). At 14 months of age those anemones that had been fed reached 15 mm in diameter and had 45 to 50 tentacles which averaged 7 mm long (Fig. 13). The column had the red patching common on the adults and the verrucae on the upper margins of the column developed white coloration. The starved ones, by contrast, had not developed the adult coloration and were still translucent white, but with an opaque white band on the tentacles; they still had only 12 to 16 tentacles; the oral discs averaged 1.2 mm in diameter (Fig. 14). In other words, they had not grown during nine months of starvation; in fact, their size had reduced.

At this writing the anemones are 18 months old. Those that have been fed have reached 4 cm in diameter with 60 to 70 tentacles. A few of the starved anemones are still alive and have not changed size appreciably.

Discussion

The identification of the various species in the genus *Tealia* is still problematic. Based on some unpublished studies of the cnidomes of the anemones of the Friday Harbor region (McIntyre, 1960, unpublished class report), we are reasonably certain in our identification. It might be useful, however, to describe briefly the animals we have studied and hopefully our observations on the development may help to elucidate the taxonomic problems. The anemone is found attached to rocks



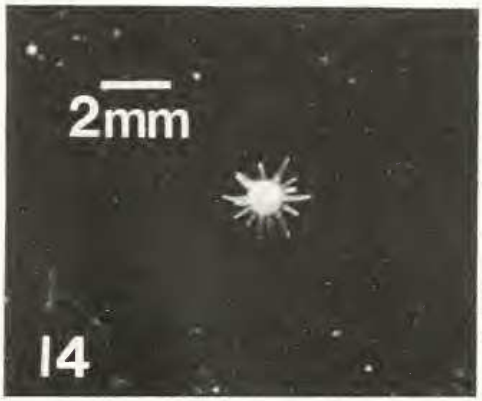


FIGURE 13. 14 month old anemone when fed in the laboratory.
FIGURE 14. 14 month old anemone, when starved for the last 9 months.

in the low intertidal and subtidal. Its base is broad (10 to 15 cm in diameter) and strongly adherent. The column is stout and cylindrical. The color varies from a yellow-brown to green and may have irregular red patches. Weakly developed verrucae may cover the column in regular rows, and are generally the same color as the column. In some specimens the verrucae on the upper margin of the column are white. No shelly or rocky materials are found adhering to the verrucae. The tentacles are short and blunt, usually light in color with one white or red band. The oral disc is usually a lighter color than the column. Red to brown lines often surround the bases of the tentacles.

The spiny egg membrane of *Tealia crassicornis* is similar to that described in other anemones. They are known to occur on the eggs of *Peachia* (Faurot, 1895),

Bolocera and *Actinia* (Gemmill, 1920, 1921) *Anthopleura, Cribrinopsis* and *Actinostola* (unpublished observations). The eggs of some other anemones are reported to have smooth membranes: *Halcampa* (Nyholm, 1949), *Metridium, Adamsia* and *Sagartia* (Gemmill, 1920). These reports of smooth membranes need verification in light of the following experience. Our first observations of the egg of *Actinostola* using bright field optics led us to call it a smooth membrane. Using phase contrast optics we observed this egg and found it to be covered by very fine spines. The spines are unique in the eggs of actiniarians. Their function is at present unknown.

Appellöf (1900) studied the embryology of *T. crassicornis* and his description of the development prior to gastrulation agrees with ours. The major difference between our interpretation and that of Appellöf is the process of germ layer formation. He described and figured an invagination process in which the central yolky material passed between the invaginated cells and came to lie in the gastrovascular cavity. The same mechanism has been reported in *Bolocera tuediae* by Gemmill (1921) and in *Actinia bermudensis* by Cary (1910). Our observations indicate that the endoderm is likely established by multipolar ingression. These cells that are moved into the interior do not form a well defined layer of endoderm until much later in development.

The formation of the so called "yolk plug" by epiboly has apparently escaped the attention of Appellöf (1900). Our study indicated that cells at the animal half proliferate rapidly and move towards the vegetal pole where cells have stopped dividing. Thus, a number of large, nondividing cells, the "yolk plug," are being overgrown and eventually are enclosed inside the embryo. The opening left from the "yolk plug" is the blastopore and later the mouth. The gastrovascular cavity (archenteron) was filled by yolk material throughout the embryonic development; it became hollow only at a much later stage (planula) by the gradual absorption of yolk.

The apical invagination described in this paper was not reported by Appellöf (1900). There is little doubt that the invaginated cells will be incorporated in the apical organ, although our histological sections of the planula larva show that the apical cells differ only slightly from other endodermal cells. Widersten (1965) did not find an apical organ in *Tealia felina*. He made the point that the apical organ is functionally important in the feeding of the larva and during the evolutionary changes from planktotrophic larvae to lecithotrophic larvae, in the phylum *Cnidaria*, the apical organ has been lost. Judging from the fact that the larvae of *T. crassicornis* will respond to different substrata and alter their time of settlement, we feel that the primary function of the apical organ is substratum selection, not feeding. Therefore, it is not surprising that the larvae of *T. crassicornis*, though lecithotrophic, possess an apical organ.

The effect of the worm tubes in promoting the settlement of larvae is not restricted to the planulae of *Tealia*. The larvae of *Cribrinopsis*, another sea anemone, respond in a similar manner. In the echinoderms, the larvae of the asteroids *Mediaster aequalis* and *Pteraster tesselatus*, and of the holothuroids *Cucumaria miniata* and *Psolus chitonoides* can be induced to settle in the presence of *Phyllochaetopterus* tubes as well (Fu-Shiang Chia, unpublished observations). The nature of the interaction is, however, unknown.

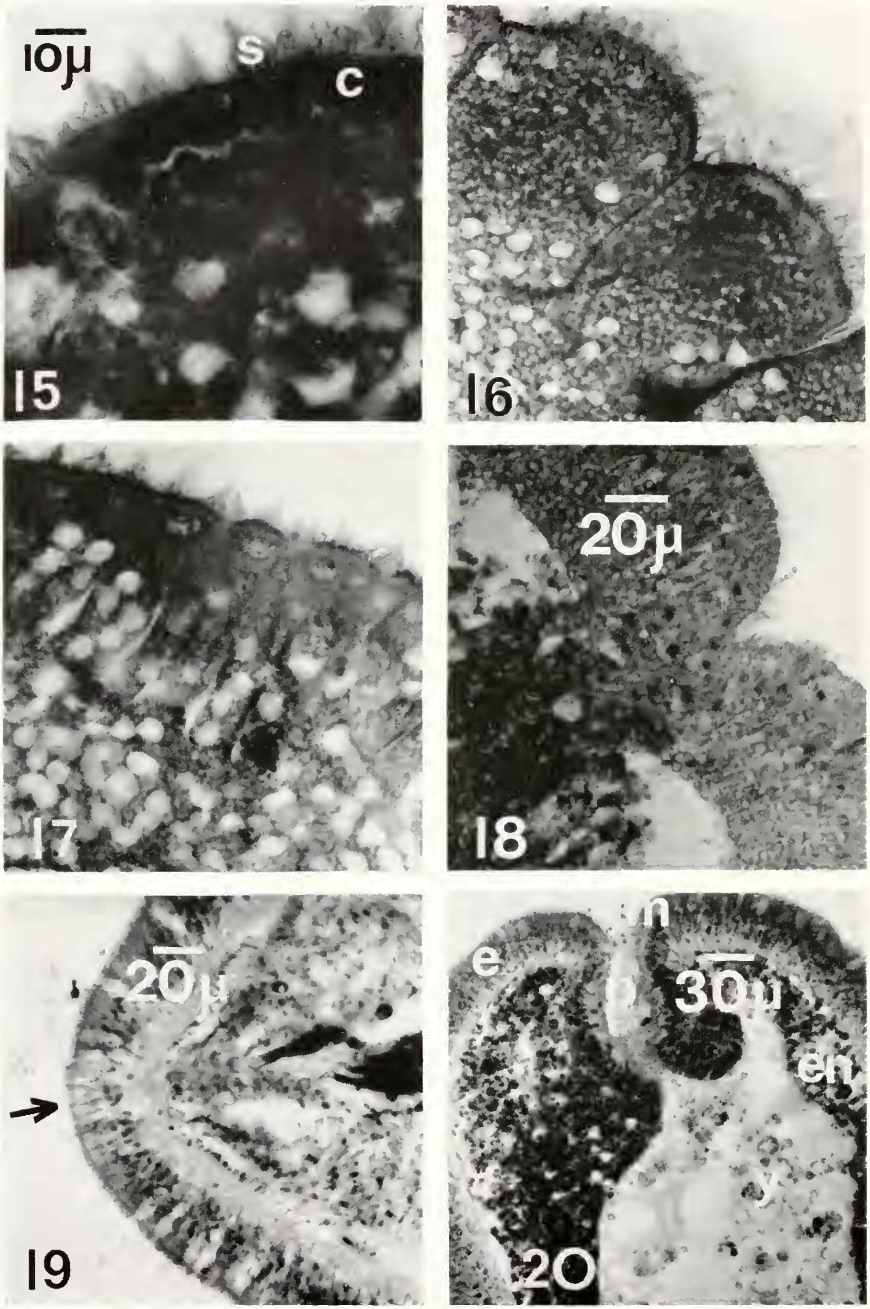


FIGURE 15. Section of a fertilized egg, showing the surface spines (s) and cortical cytoplasm (c).

FIGURE 16. Section of an early cleaving embryo, showing the incomplete cell membrane.

The growth of the juvenile, as is shown in our study, is totally dependent upon the amount of food it takes, and hence the size of the animal has little to do with age. It is interesting to note that the young anemones are able to withstand at least nine months and possibly a much longer period of starvation. The starved animal not only did not grow but decreased in size. During this period they may be directly utilizing dissolved organic molecules from sea water or they may feed on micro-organisms for maintaining themselves.

Based on the differences of the growth rate between well-fed and starved animals as we have observed, it is impossible to speculate upon the age of a typical reproductive adult of *T. crassicornis* (10 to 15 cm in diameter). It is certain, however, that more than one year is required to reach the reproductive condition since our well-fed specimens show no sign of gonad development at 14 months.

The fact that *T. crassicornis* are brooders in some areas and shed gametes freely into the sea at Friday Harbor may either be due to the fact that the two populations are two different species, or that they are the same species but differ in reproductive behavior. The plasticity in the mode of reproduction exists also in other sea anemones such as *Actinia equina* as discussed by Chia and Rustron (1970), and *Sagartia troglodytes* discussed by Stephenson (1929).

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Summary

1. *Tealia crassicornis* in the Friday Harbor Laboratories was observed to spawn from April to June. The eggs measure 500–700 μ in diameter, and bear surface spines 25 μ in length.

2. The cleavage is superficial, resulting in small cells on the animal pole and large cells on the vegetal pole.

3. Endoderm appears to be formed by multipolar ingression while the central yolk mass remains uncleaved and the formation of the gastrovascular cavity is achieved by absorption of yolk material.

4. The settlement of planula larvae can be facilitated by adding the tubes of the polychaete worms, *Phyllochaetopterus* or *Sabellaria* to the culture dishes.

5. A chronology of development is presented and the histogenesis of larval tissue is described.

Figure 17. Section of a late cleaving embryo, showing the superficial layer of cells and the uncleaved yolk mass.

Figure 18. Formation of apical organ by invagination of ectoderm at the apical end of a gastrula, the space between the ectoderm and the endoderm and yolk mass in this photograph is an artifact.

Figure 19. Sagittal section of a planula larva showing the apical group of cells with clear cytoplasm (arrow).

Figure 20. Sagittal section of a planula larva showing the mouth (m), pharynx (p), ectoderm (e), endoderm (en), and the remnant of the yolk mass (y).

6. The young anemones have grown to a size of 4 cm in diameter with 60 to 70 tentacles within 18 months when fed in the laboratory.

7. Young anemones can withstand starvation for at least 9 months. There is no growth when starved.

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